Response of Apple Buds Cultured in Vitro to Abscisic Acid

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Additional index words. Malus domestica, bud break, shoot elongation, hydrolyzable-ABA

Abstract. Abscisic acid (ABA) inhibited bud break and shoot elongation in seedling stem explants of apple (Malus domestica Borkh cv. Northern Spy) cultured in vitro. Inhibition was complete in culture medium containing 100 \(\mu\)M ABA. Transfer of buds from ABA-containing medium to basal medium resulted in increases in both bud break and shoot elongation. ABA levels in such buds declined rapidly following transfer, and growth began when ABA concentration in the buds dropped below a threshold value.

A delay in bud break following ABA applications has been reported in dormant apple seedlings (8) and in rose and lilac (3). ABA also inhibits bud break in vitro in citrus (2) and apple (4). Besides delaying bud break, ABA causes the cessation of shoot elongation (11) and the formation of terminal buds in apples (8, 10) and several other plants (5). However, discontinuing ABA applications results in a rapid loss of the ABA-induced inhibition of bud break and shoot elongation (2, 8, 11).

This investigation was conducted to determine the effects of ABA on the growth of apple buds and the relationship between bud break and the level of exogenously applied ABA in the buds. The technique of in vitro culture of apple bud explants (4) was employed because individual buds, isolated from other growth interactions, can be studied under controlled environmental conditions after ABA has been applied at known concn for predetermined periods of time.

Materials and Methods

All experiments were conducted with vegetative axillary buds from the mid-shoot region of greenhouse grown 'Northern Spy' seedlings 30-50 cm in height. After removing the leaves, the intact bud stick was surface sterilized by soaking for 15 min in 0.75\% sodium hypochlorite. This bud stick was then cut into segments such that each explant consisted of one bud with 0.5-1 cm of stem above and 1.0-1.5 cm of stem below the bud. The detailed procedure for in vitro apple bud culture has been reported by Dutcher and Powell (4). Bud explants were cultured on 10 ml of modified Murashige and Skoog medium developed for apple tissues (4) and solidified with 0.75\% agar. This complete medium, without ABA, is hereafter referred to as basal medium. The (+) cis-trans isomer of ABA was incorporated into this medium prior to autoclaving for 20 min at 120°C. The cultures were maintained under constant illumination provided by a 1:1 combination of daylight and Gro-lux fluorescent tubes.

Effect of ABA on bud break. Twelve explants were initially cultured in media containing 0, 10, 50 or 100 \(\mu\)M ABA for 5 days and then transferred to basal medium. Two cultures contaminated during transfer were discarded. Bud break and shoot length were determined 18 days after transfer.

Effect of short duration ABA treatment on bud growth. Twelve explants were initially cultured in media containing 0, 10, 50 or 100 \(\mu\)M ABA for 5 days and then transferred to basal medium. Two cultures contaminated during transfer were discarded. Bud break and shoot length were determined 18 days after transfer.

Metabolism of ABA in vitro. Explants were cultured for a period of 4 days on media containing 0, 10 or 100 \(\mu\)M ABA and then transferred to basal medium. Starting on the first day after transfer, samples of 5 buds or the shoots resulting therefrom were excised for ABA analysis. These were immediately frozen in liquid nitrogen, then lyophilized. The samples were weighed, then extracted by shaking in 80\% methanol for three 2-day periods at 2-3°C in the dark, changing the methanol every 2 days. Free and hydrolyzable-ABA were determined as previously described (9). After methylation with diazomethane, aliquots were injected into a Barber-Coleman gas chromatograph equipped with an electron capture detector and a column (3 mm i.d. x 2 m) packed with 1\% GE XE-60 on Anachrom-ABS support. Injection port, column and detector temp were 220°, 195° and 230° respectively.

Results

The effect of ABA on bud break and shoot elongation. Bud break and shoot length declined with increasing ABA concn, inhibition being complete at 100 \(\mu\)M (Fig. 1). Prolonged exposure, especially to 100 \(\mu\)M ABA, frequently resulted in explant injury or death. Injury to the explants, as indicated by yellow or brown coloration of the culture medium, was evident in some of the explants in the 10 \(\mu\)M treatment. Callus formation tended to increase with increasing ABA concn and was pronounced at 100 \(\mu\)M. The callus developed on the upper end of the explant and/or around the bud, and occasionally on the end embedded in the medium.

The effect of short duration treatment on bud growth. When explants were cultured on medium containing ABA and then transferred to basal medium both bud break and shoot length increased (Table 1). This transfer also reduced explant injury. However, bud break and shoot length did not approach that of explants not supplied with ABA.

Metabolism of ABA in vitro. Transferring explants from ABA-containing medium to basal medium resulted in a rapid decline in ABA content of the buds (Fig. 2). The control buds (from explants started in basal medium and transferred to similar medium after 4 days) contained virtually no measurable ABA and bud break was evident 6 days after transfer. In the 10 \(\mu\)M treatment, bud break did not occur until 10 days.

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Fig. 1. Effect of ABA on bud break and shoot length in apple bud explants after 21 days in media containing ABA. Each point is the mean for 2 experiments. Control: bud break = 100%, shoot length = 1.9 ± 0.1 cm. Vertical bars indicate standard error of mean.

After transfer. By this time the ABA level had declined to the control level (Fig. 2). Development of buds transferred from 100 µM medium was not evident until day 14. Although insufficient tissue was available for ABA analysis at this time, the ABA concn was probably approaching that of the control (Fig. 2).

Endogenous ABA levels increased when shoot elongation commenced. Control buds contained no measurable ABA until 6 days after transfer. By day 10 all control buds had begun to grow. The shoots contained 0.46 ng/mg ABA at this time and 0.43 ng/mg on day 12. Twelve days after transfer from 10 µM to basal medium non-growing and growing buds contained 0.18 and 0.39 ng/mg ABA respectively.

High levels of hydrolyzable — ABA were observed in buds after transfer from ABA — containing medium to basal medium (Fig. 2). This concn gradually declined during the 12 days following transfer.

Table 1. The effect of short duration ABA treatment on bud break and shoot length.2

<table>
<thead>
<tr>
<th>ABA concn (µM) in which buds were explanted</th>
<th>ABA concn (µM) to which buds were transferred</th>
<th>Bud break (%)</th>
<th>Shoot length (% control)</th>
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<tr>
<td>100</td>
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<td>10</td>
<td>0</td>
<td>66</td>
<td>28</td>
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2Treated buds were cultured for 5 days on medium containing ABA, then transferred to basal medium. Results recorded 18 days after transfer. Values for controls (no ABA): bud break = 100%, shoot length = 2.73 ± 0.2 cm.

Fig. 2. Changes in ABA concn in buds of apple seedling explants cultured in 0, 10 or 100 µM ABA for 4 days and then transferred to basal medium. Values are means for 2 samples, each containing 5 buds or the growth therefrom. Vertical bars indicate standard error of mean.

Discussion

The incorporation of ABA into the culture medium inhibited both bud break and shoot elongation. The concn of ABA required to prevent bud growth were relatively high. Similar responses have been reported in other in vitro studies (2, 4). The promotion of callus formation following ABA application has also been observed in citrus and apple explants in vitro (1, 6) and on aspen root cuttings (12).

Upon transferring explants from medium containing ABA to basal medium, bud break and shoot elongation increased. A
rapid loss of the inhibitory effect of ABA was also observed in citrus cultures (2) and in intact apple plants (8, 11). The level of ABA in the buds declined rapidly following transfer (Fig. 2). Bud break in control explants occurred within 6 days of transfer, but not until 10 days in explants initially treated with 10 \( \mu M \) ABA. Once the level of exogenously applied ABA in the buds declines below a threshold level the inhibitory effect apparently is lost and growth can occur.

The level of hydrolyzable-ABA was high one day after transfer of explants from ABA-containing medium to basal medium, indicating that this was a product of ABA inactivation. However, its subsequent decline indicates that either it diffused into the culture media or that other inactivation products (13) were being formed.

The concn of endogenous ABA in both the control buds and in buds which had initially been treated with 10 \( \mu M \) ABA increased as growth began. This increase in ABA, paralleling the initiation of shoot elongation, lends further support to the proposal that ABA in itself is not the dominant hormone in the cessation of shoot growth (7).

Our primary interest was to relate the levels of ABA in the buds to bud break. We postulate that the level of ABA in the bud must be maintained above a threshold value if bud break is to be inhibited. Based on a rapid decline of applied ABA in this and a previous study (9) we conclude that a prolonged delay of bud break cannot be achieved in the absence of a continuous supply of ABA.

In an in vitro system, where the buds are isolated from outside sources of growth promoting hormones and other substances, the response to ABA appears to be more marked than in intact buds. Caution must therefore be exercised in drawing parallels between in vitro and intact systems.

Literature Cited


Fruit Turgor Influences Susceptibility of ‘Tahiti’ Lime to Stylar-end Breakdown1

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Additional index words, stylar-end rot, Citrus latifolia, fruit water potential, rind oil release pressure

Abstract. 'Tahiti' (Citrus latifolia Tan.) lime fruit varying in turgor, estimated by fruit water potential and rind oil release pressure, were collected and assayed for susceptibility to stylar-end breakdown. Susceptibility was determined by heating fruit for 3 hours at 42°C in a constant temperature water bath. Incidence of stylar-end breakdown was about 40% in the most turgid fruit, water potential >—4 bars and rind oil release pressure ≪2.0 kg. Decreasing turgor resulted in a linear decrease in susceptibility. Minimum susceptibility of from 0% to 2% was found in limes with water potential ≪—11 bars and rind oil release pressure ≫4.5 kg. Stylar-end breakdown can be controlled by maintaining strict picking schedules so that fruit are not allowed to get too large, controlling post-harvest field heat, and harvesting fruit with reduced turgor pressure.

Stylar-end breakdown (SEB), a postharvest disorder of ‘Tahiti’ lime fruit, has caused significant losses to the Florida lime industry for many years (3). Packinghouse grade-outs attributed to SEB vary throughout the year with losses greater than 40% frequently occurring in the hot summer months (4, 5, 17). Symptoms of SEB have been described (13, 17). Davenport and Campbell (5) observed that SEB symptoms involve traumatic breakage of juice vesicles located in the periphery of fruit locules. Release of juice from these vesicles, followed by juice invasion of the rind via the central core was thus responsible for localized breakdown of rind tissue symptomatic of the disorder.


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